

FIG. 1A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS					
		1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
Thr*	X-X-X-X-Thr-X-X-X-X-X-Cys	1.92	1.32	0.54	0.34	0.07	0.04
Ser-Thr	X-X-X-X-X-Ser-Thr-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01
Akt-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Phe-Cys-Gly-Thr-Pro (SEQ ID NO:1)	1.18	0.85	0.24	0.13	0.03	0.01
APPI1-Thr688-P	Asp-Ala-Ala-Val-Thr-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys (SEQ ID NO:2)	0.14	0.03	0.01	0.01	0.01	0.01
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Asn-Thr-Phe-Val-Gly-Thr-Pro-Phe-Cys (SEQ ID NO:3)	1.71	1.13	0.39	0.22	0.03	0.02
CAK-Thr167-P	His-Gln-Val-Val-Thr-Arg-Trp-Tyr-Arg-Cys (SEQ ID NO:4)	1.77	1.15	0.41	0.27	0.06	0.03
CAMIV-Thr186-P	His-Gln-Val-Leu-Met-Lys-Thr-Val-Cys-Gly (SEQ ID NO:5)	1.79	1.36	0.63	0.40	0.09	0.05
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Tyr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:6)	1.02	0.56	0.14	0.08	0.03	0.01
CDK2-Thr159-P	Gly-Val-Pro-Val-Arg-Thr-Tyr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:7)	1.88	1.79	0.51	0.44	0.08	0.04
p70S6K-Thr289-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO:8)	1.99	1.44	0.82	0.39	0.08	0.04
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO:9)	1.82	1.83	0.94	0.58	0.15	0.08
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Val-Ala-Thr-Arg-Trp-Cys (SEQ ID NO:10)	1.56	1.18	0.51	0.30	0.07	0.04
Myo Ser58/62-P	Glu-Leu-Leu-Pro-Thr-Pro-Pro-Leu-Ser-Pro-Ser-Arg-Arg-Ser-Cys (SEQ ID NO:11)	0.11	0.05	0.03	0.02	0.02	0.02
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Asp-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Arg-Cys (SEQ ID NO:12)	0.54	0.30	0.08	0.08	0.04	0.04
JNK2P	Ser-Phe-Met-Met-Thr-Pro-Tyr-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys (SEQ ID NO:13)	1.49	0.44	0.12	0.07	0.03	0.02

100 111 121 131 141 151 161 171 181 191

FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXXS*XXXXXX	—
XXXXY*XXXX	—
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	++
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—
XXXXRSXSXPXXXX (SEQ ID NO:16)	—
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	++
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXXT*XXXXXX	+++
XXXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+++
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
NEB LIBRARY	
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+++
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	++
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+++
X-X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+++
X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23)	+++
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+++
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-X-C (SEQ ID NO:25)	+++
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26)	++
X-X-X-X-X-T*-X-X-X-X-X-X-C	+++
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	++
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-X-C (SEQ ID NO:29)	++
ANTIBODY REACTIVITY	
+++ very strong	ELISA O.D. > 2
++ strong	1 - 2
+ weak	0.2 - 1
- very little	< 0.2

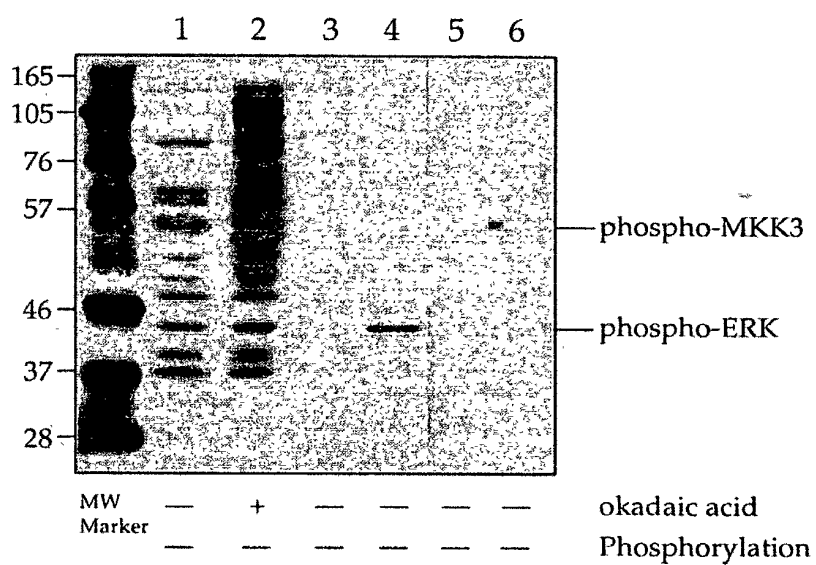


FIG. 1D

Fixed Amino Acid	-5-4-3-2-1 X X X X X Ser*/Thr* X X X X X Fixed AA position relative to phospho-Ser*/Thr*								
	- 4	- 3	- 2	- 1	S*/T*	+ 1	+ 2	+ 3	
Ala	+	+	+	+		+	+	+	+
Cys	+	+	+	+		+	+	+	+
Asp	+	+	+	+		+	+	+	+
Glu	+	+	+	+		+	+	+	+
Phe	+	+	+	+		+	+	+	+
Gly	+	+	+	+		+	+	+	+
His	+	+	+	+		+	+	+	+
Ile	+	+	+	+		+	+	+	+
Lys	+	+	+	+		+	+	+	+
Leu	+	+	+	+		+	+	+	+
Met	+	+	+	+		+	+	+	+
Asn	+	+	+	+		+	+	+	+
Pro	+	+	+	+		—	+	+	+
Gln	+	+	+	+		+	+	+	+
Arg	+	+	+	+		+	+	+	+
Ser	+	+	+	+		+	+	+	+
Thr	+	+	+	+		+	+	+	+
Val	+	+	+	+		+	+	+	+
Trp	+	+	+	+		+	+	+	+
Tyr	+	+	+	+		+	+	+	+

10017465 .031002

FIG. 2A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS					
		1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
PKSP-P	X-X-X-X-X-Pro-X-Ser-Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 27)	1.82	1.97	1.74	1.40	0.70	0.35
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.67	0.36	0.13	0.07
SerThr	X-X-X-X-X-SerThr-X-X-X-X-X-Cys (SEQ ID NO: 28)	0.14	0.03	0.01	0.00	0.00	0.00
FB Thr373-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Aug-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)	2.07	2.17	1.70	1.20	0.48	0.18
MKK3-Thr-P	Ser-Val-Ala-Lys-Thr-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)	0.06	0.04	0.01	0.00	0.00	0.00
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO: 9)	0.05	0.02	0.01	0.00	0.01	0.00
p70 S6K-Thr389	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 8)	0.11	0.05	0.01	0.00	0.01	0.00
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr-Pro-Val-Val-Lys-Cys (SEQ ID NO: 32)	2.07	2.21	2.01	1.55	0.69	0.31
							0.07

1.00E+03 5.00E+03 1.00E+04 5.00E+04 1.00E+05 5.00E+05

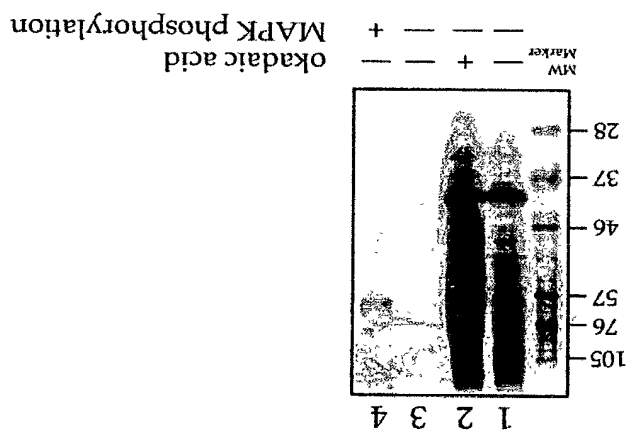


FIG. 2B

100-443886-1036

100-443886-1036

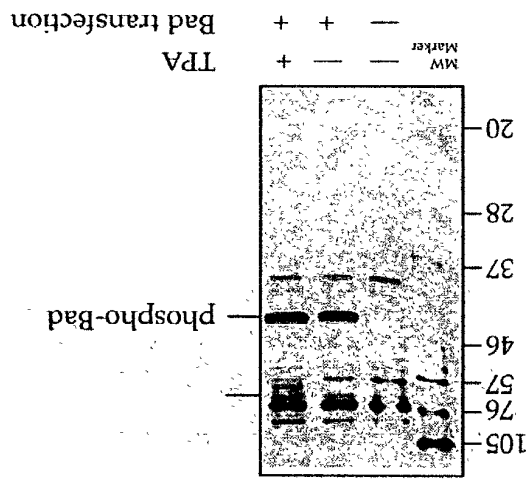


FIG. 3B

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

FIG. 4A

PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES	
		6B8	5A9
Ser/ThrPro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:26)	1.774	0.731
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr*-Pro-X-X-X-X-X-Cys (SEQ ID NO:27)	0.924	0.766
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:41)	0.02	0.063
ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser/Thr*-Pro-X-Arg/Lys-X-X-X-Cys (SEQ ID NO:42)	1.955	1.275
Thr-P	X-X-X-X-X-X-Thr*-X-X-X-X-X-X-Cys	0	--
Ser-P	X-X-X-X-X-X-Ser*-X-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-X-Tyr*-X-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO:43)	0.032	0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:30)	3.336	3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:44)	0.02	0.073

138 CIP2, Sheet 9 of 25

FIG. 4B

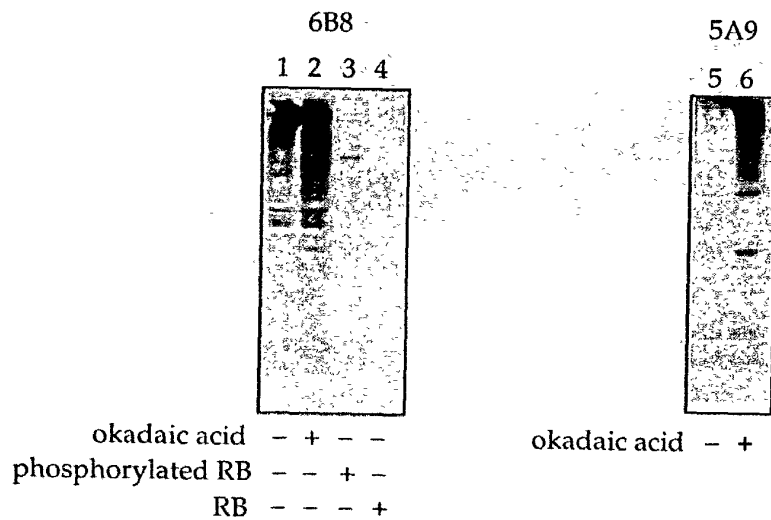


FIG. 5A

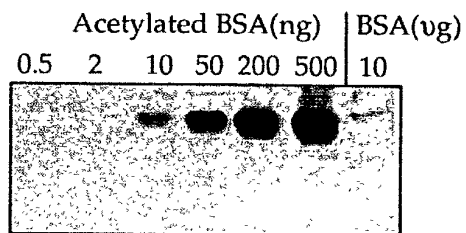


FIG. 5B

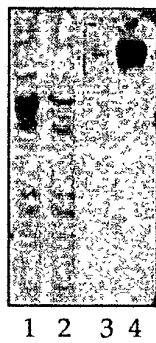


FIG. 5C



FIG. 5D

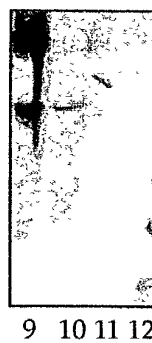


FIG. 6

Signal to noise ratio of ELISA
 readings using phospho-Akt substrate
 antibody.

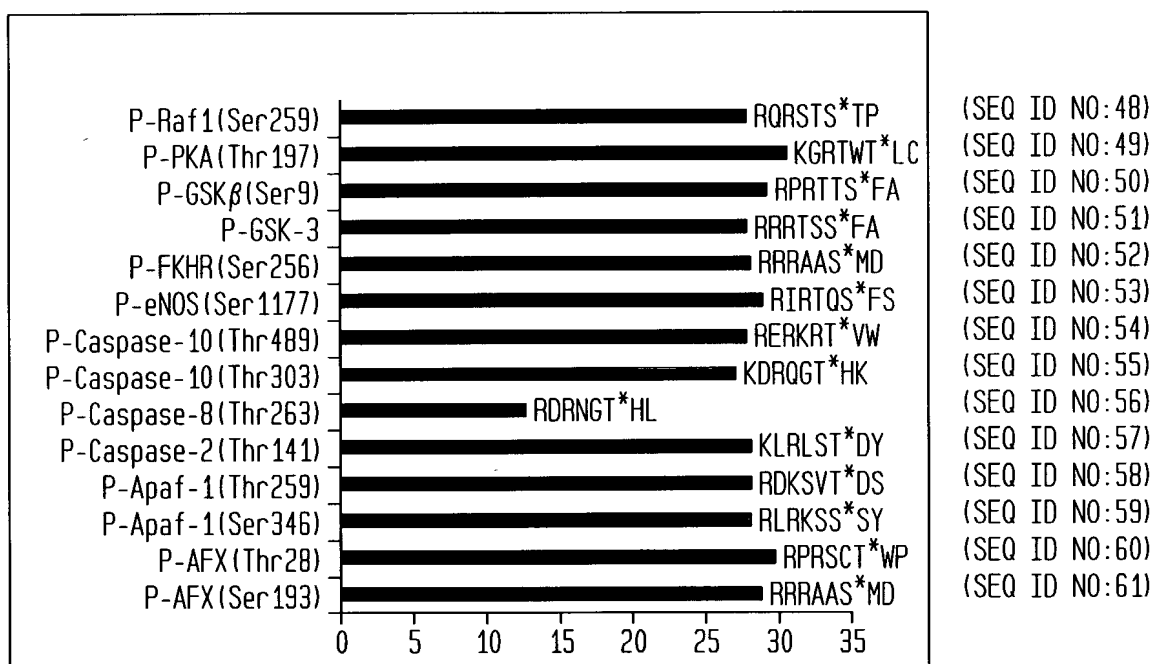
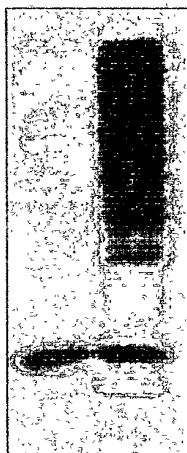


FIG. 7

Western analysis of
calyculin A-treated A431 cells
using Phospho-Akt Substrate Anti-
body.



- +
calyculin A

FIG. 8

Signal to noise ratio of ELISA
reading using phospho-PKA substrates
antibody.

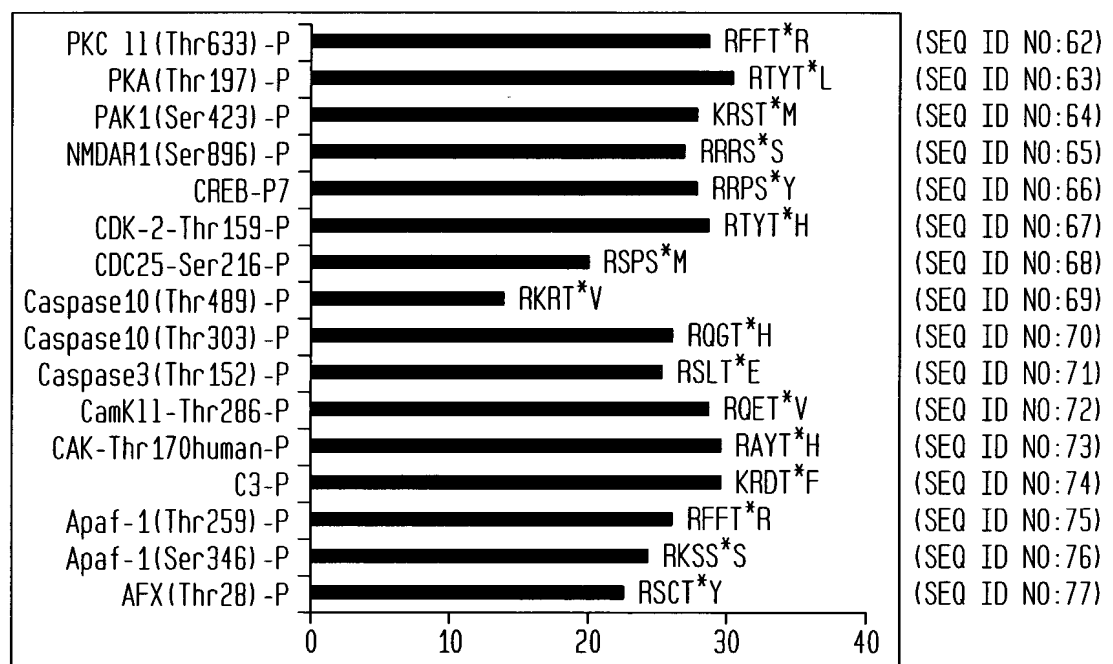




FIG. 9

Western analysis of
calyculin A-treated A431 cells
using Phospho-PKA Substrate
Antibody.

- + calyculin A

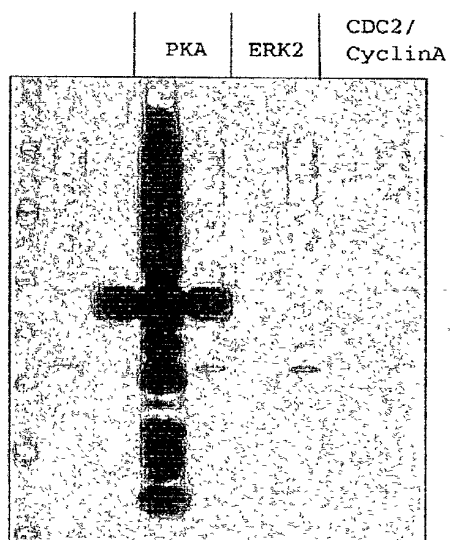


FIG. 10

Western analysis of
A431 cell extracts

+	-	+	+	-	+	+	Cell Extracts
-	-	-	+	-	-	-	PKI

FIG. 11

Signal to noise ratio of ELISA
reading using phospho-Serine/threonine
phenylalanine antibody.

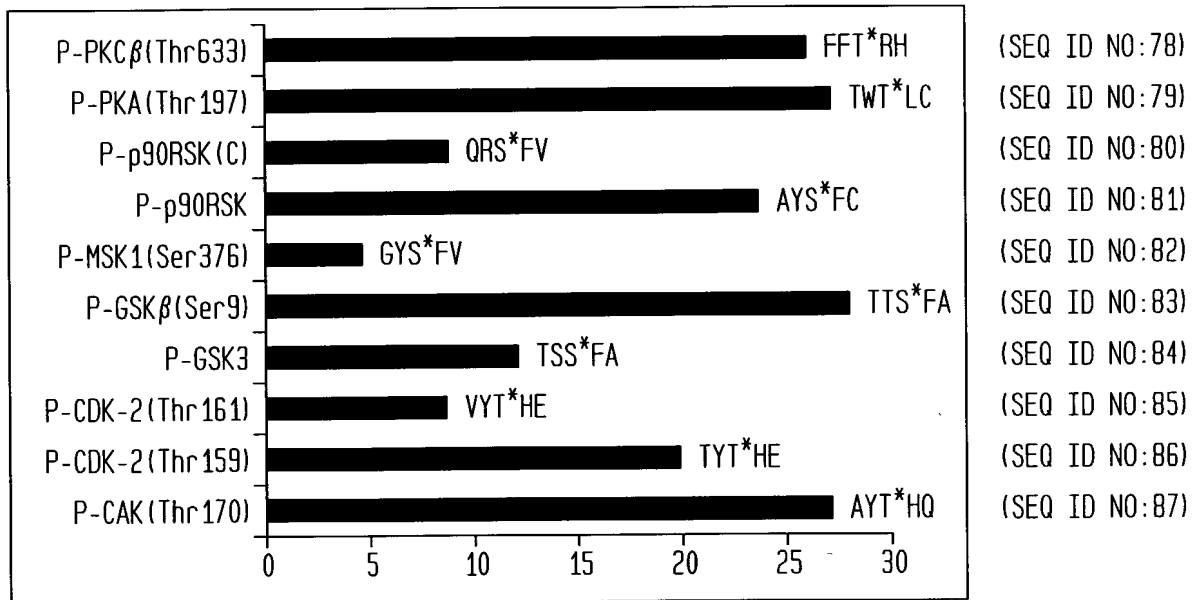


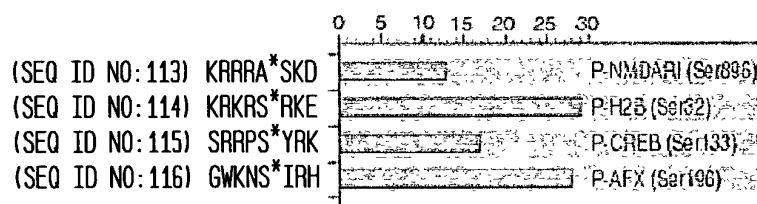
FIG. 12

Western analysis of calyculin
A-treated A431 cells using phospho-
Serine/phenylalanine substrates antibody.



- +
calyculin A

Signal to noise ratio of ELISA reading using a context-independent phospho-PKC consensus substrate motif antibody.



Western blot analysis using a context-independent phospho-PKC consensus substrate motif antibody.

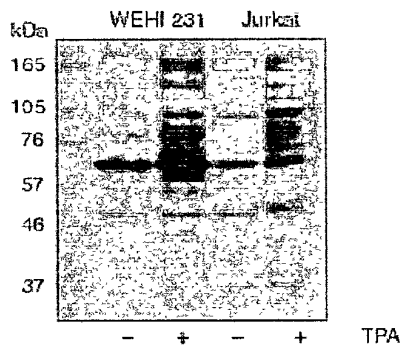


FIG. 15

Western blot analysis of whole cell lysates using a context-independent antibody specific for the phospho-PKC consensus substrate motif.

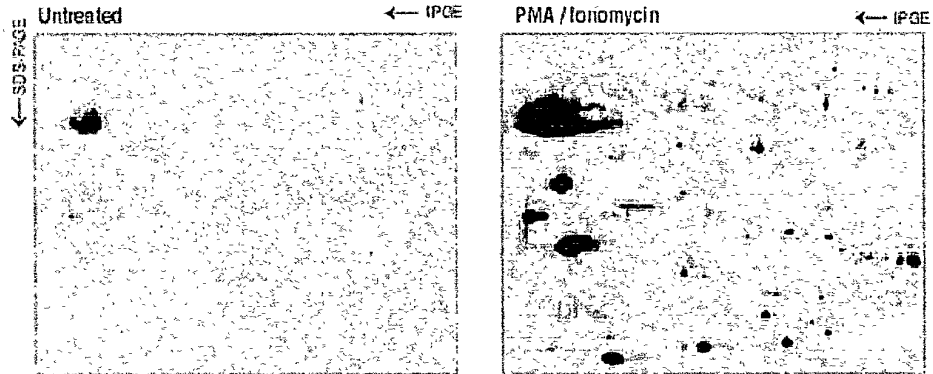


FIG. 16

Western analysis of whole cell lysates using a phosphotyrosine and nitrotyrosine - specific context-independent antibodies.

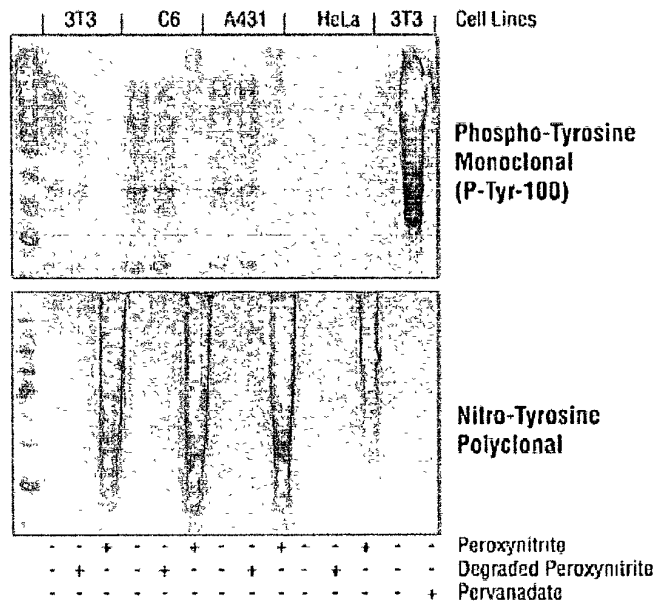


FIG. 17

Immunocytochemical staining of NIH/3T3 cells using a polyclonal context-independent antibody specific for nitrotyrosine (brown).

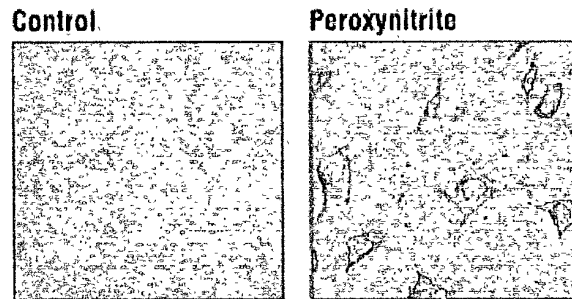


FIG. 18

Phosphothreonine-X-arginine motif-specific context-independent antibody ELISAs.

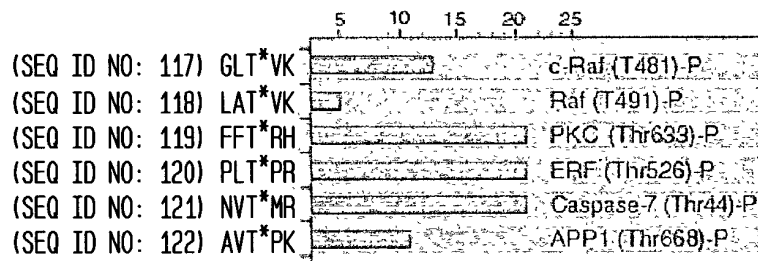


FIG. 19

Western blot analysis of Jurkat cell extracts using a context-independent antibody specific for the phosphothreonine-X-arginine motif.

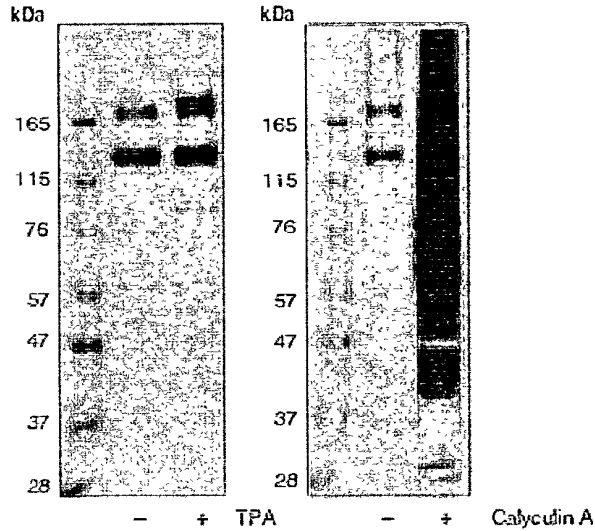


FIG. 20

IHC staining of proteins containing phosphorylated threonine-X-arginine motifs in human breast carcinoma.

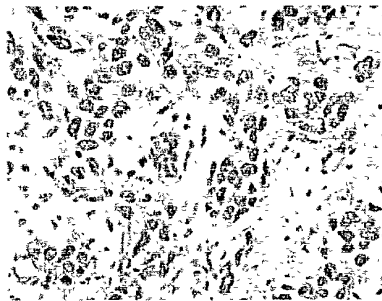


FIG. 21

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibody specific for the phospho-14-3-3 binding motif #2 (phospho(Ser)-Arg-X-(Tyr/Phe)-X-pSer).

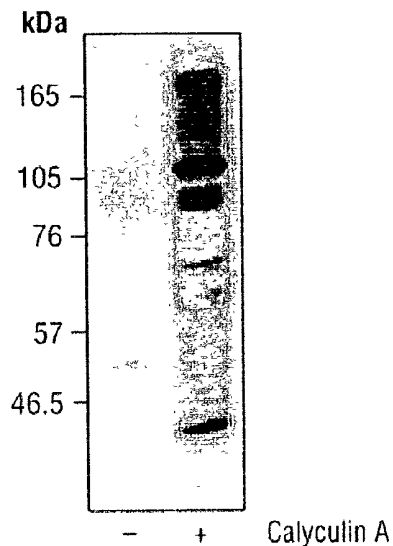
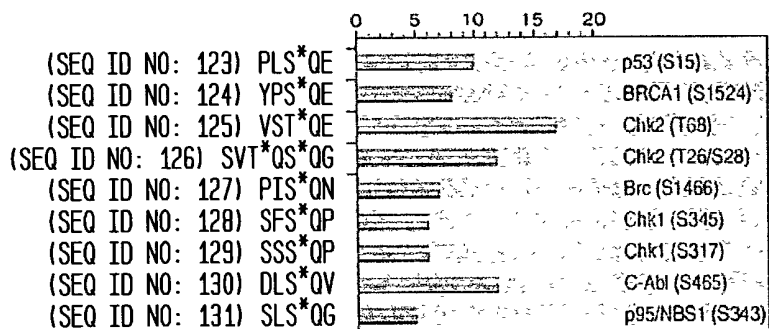


FIG. 22

Phospho-ATM/ATR consensus substrate motif-specific, context-independent antibody ELISAs.



Western blotting of COS cell extracts using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.



Western blot analysis of UV treated COS cells, using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.



Phospho-14-3-3 binding motif-specific, context-independent monoclonal antibody ELISAs (T* and S* denote phospho-threonine and serine).

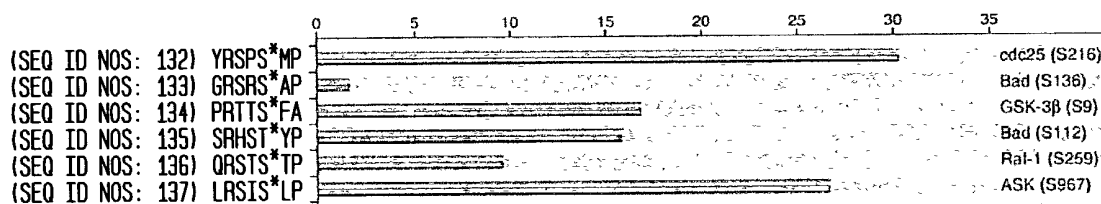


FIG. 26

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibodies specific for phospho-14-3-3 binding motif #1.

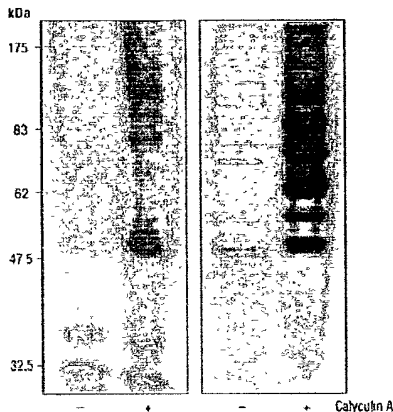


FIG. 27

Phospho-PDK1 docking motif-specific, context-independent monoclonal antibody ELISAs. (T* and S* denote phospho-threonine and serine.)

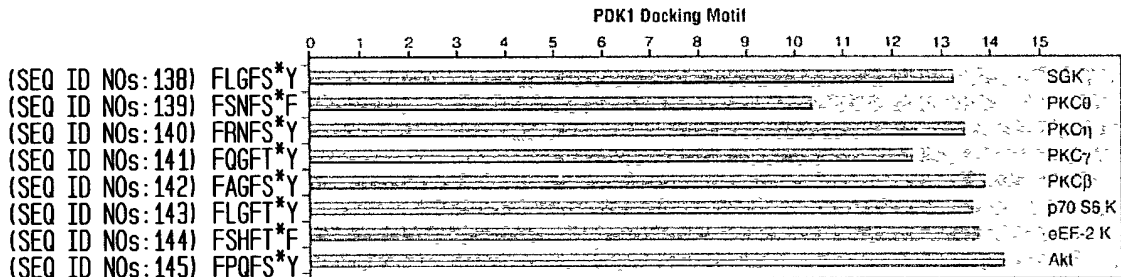


FIG. 28

Western blot analysis of extracts from A431 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

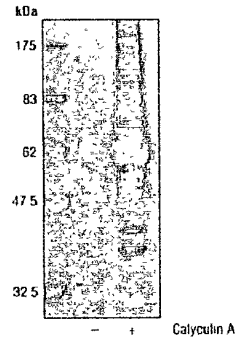


FIG. 29

Immunoprecipitation of extracts from NIH/3T3 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

